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Detection of *Proteus mirabilis* as foodborne disease bacteria in carcass of broiler chickens (*Gallus domesticus*)

Abdul Wahid Jamaluddin^{1*}, Lucia Muslimin¹, Muhammad Natsir Djide²

¹Study Program of Veterinary Medicine, Faculty of Medicine, Hasanuddin University, Jl. Perintis Kemerdekaan Km. 10, Makassar 90245, Indonesia

²Faculty of Pharmacy, Hasanuddin University, Jl. Perintis Kemerdekaan Km. 10, Makassar 90245, Indonesia

*Corresponding author: Abdul Wahid Jamaluddin (abdulwahidjamaluddin@unhas.ac.id)

Abstract

Poor hygiene of traditional market often resulted in bacterial contamination of chicken meat including *Proteus mirabilis* which is also serve as a foodborne disease in human. *Proteus mirabilis* is pathogenic to humans because it can produces the urease which results in urinary tract infections (UTI). This research aimed to determine the presence of *Proteus mirabilis* bacteria in chicken meat sold in some traditional markets in the city of Makassar. The samples used in this study (24 samples) were obtained from 6 Traditional Markets in Makassar. Colony observation, Gram staining and biochemical test were used to identify *Proteus mirabilis* in the chicken carcass. Positive samples were confirmed by Polymerase Chain Reaction (PCR). The result of this study revealed that 12.5% (3/24) were positively contaminated with *Proteus mirabilis*. The conclusion of this study was that chicken meat sold in the traditional market of Makassar has already been contaminated with *Proteus mirabilis*.

Keywords: *Proteus mirabilis*, foodborne disease, urinary tract infection, carcass chicken, traditional market

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Introduction

Foodborne disease is caused by consuming food or water contaminated (Sukron, 2011) and this is a part of zoonosis. Zoonosis is an infectious disease which can be transmitted from animals or products of animal origin to humans (Muslimin et al., 2014). Research conducted by Amare et al. (2013) revealed that 66 out of 290 hens were positive of *Proteus mirabilis* with an infected yolk sac found in the postmortem examination. *Proteus mirabilis* is pathogenic to human because it can lead to urinary tract infection (UTI), which is the most common clinical manifestations of infection *Proteus sp.* (Gonzalez et al., 2014).

Antibiotic resistance to *Proteus mirabilis*, isolated from chicken in China resulted as follows: Tetracycline (100%), sulfamethoxazole (80%), chloramphenicol (66%), nalidixic acid (66%), ampicillin (36%), cefotaxime (34%), ceftiofur (22%), amoxicillin and clavulanic acid (36% 16%) (Wong et al., 2013). Another case in India is semi-resistant/ moderately susceptible to antibiotics from *Proteus mirabilis* isolates derived from beef are Penicillin G, erythromycin, amoxiclav, Cefoxitin and Cotrimoxazole (Gupta et al., 2014). On the other hand, the use of

antibiotics now used to prevent and treat infections caused by bacteria in humans and livestock is now a great concern for their resistance resulting in failure of the treatment of infections diseases caused by pathogenic bacteria in humans and increased costs of treatment (Noor et al. 2006). This research aimed to determine the presence of *Proteus mirabilis* bacteria in chicken meat sold in some traditional markets in the city of Makassar.

Materials and Methods

Twenty-four chicken meat samples were taken randomly from six traditional markets in Makassar. Total Plate Count (TPC) test was conducted by inserting 1 gr of sample into a sterile plastic and then crushed with a mortar. Samples were added by aquadest 9 ml aseptically, then inserted into the tube and labeled.

The 10^{-1} dilution sample described above was put into 3 ml Tryptone Soya Broth (TSB) for isolation, then incubated for 24-48 hours at 37°C. Bacterial suspension in TSB which has been incubated was scratched into the Mac Conkey Agar (MCA) and then incubated for 18-24 hours at 37°C. Bacterial colony from MCA was taken and stained by Gram staining, followed by biochemical tests. *Proteus mirabilis* ATCC 43 071 was used as positive control. Biochemical test included TSIA, SIM, MR / VP, citrate, urea, catalase test, and 4 tests such as glucose, lactose, sucrose, and mannitol. Samples that have a similar biochemical profile to control *Proteus mirabilis* ATCC 43 071 were then processed with PCR.

The primer used in this study was designed to identify the coding DNA sequence of the *Proteus mirabilis* gene as 5-CCGGAAC AGAAGTTGTC GCTGGA -3' for forward and 5-GGCT CTCC TACC GACT TGATC-3' for reverse (PT. Genetic Science Indonesia, Jakarta, Indonesia) with a length of 532 bp. The PCR reaction was conducted under the following condition; denaturation cycle at 95°C for 15 min then 94 °C about 1 min, annealing at 63 ° C for 30 sec, extension at 72 ° C for 1 min followed by 40 cycles and final extension at 72 °C for 7 min and 12 ° C \pm 30 min for storage. The size of the DNA fragments PCR amplification product *Proteus mirabilis* was compared to the size of the DNA marker (Marker) to know the size of the target DNA (bp). Positive result was indicated by the band at 532bp size.

Results and Discussion

Based on the data in Table 1, there were 4 samples from 3 markets exceeded the maximum limit of microbial contamination in chicken meat determined by BSN (1×10^{-6} CFU / g). This is possibly due to the traditional market conditions of selling chicken meat openly and the location of markets that are on the busy roadside, possibly increased the microbial contamination. Based on the profile of bacterial colonies on MacConkey agar, Gram staining and biochemical tests on 24 samples / colorless colonies on MacConkey from 6 traditional markets in the city of Makassar, there are 8 samples (33%) were positively identified with *Proteus sp* (Table 2).

Table 1. Results of Chicken Meat TPC at 6 Makassar City Traditional Market

No	Place of sampling	Number samples Of	Sample Code	TPC CFU/g
1.	Market D	4	D1	4,6x10 ⁵
			D2	4,2x10 ⁵
			D3	4,4x10 ⁵
			D4	3,3x10 ⁵
2.	Market S	4	S1	1,4x10 ⁵
			S2	1,7x10 ⁵
			S3	1,6x10 ⁵
			S4	2,2x10 ⁵
3.	Market B	4	B1	2,4x10 ⁵
			B2	2,8x10 ⁵
			B3	1,4x10 ⁵
			B4	1,3x10 ⁵
4.	Market TL	4	TL1	11x10 ⁵
			TL2	11,2x10 ⁵
			TL3	11,4x10 ⁵
			TL4	10,8x10 ⁵
5.	Market PT	4	PT1	19x10 ⁵
			PT2	21x10 ⁵
			PT3	18,3x10 ⁵
			PT4	22,5x10 ⁵
6.	Market MC	4	MC1	18,3x10 ⁵
			MC2	22,5x10 ⁵
			MC3	20x10 ⁵
			MC4	18x10 ⁵

* Limit of Microbial Contamination (BMCM) > 1x10⁻⁶ CFU / g (BSN. 2009)

Proteus sp such as *Proteus mirabilis* was colorless on Mac Conkey agar because this bacteria did not ferment the lactose. *Proteus sp* which is a gram-negative bacteria are red (by safranin color given on the last stage) when stained by Gram staining due to its thin peptidoglycan wall, so it is easily removed when washed with alcohol and was not be able to retain the purple color of crystal violet as described in Figure 1 and 2.

The result of biochemical test of *Proteus mirabilis* in TSIA showed a slant pink and butt black appearance due to the ability of this bacteria to ferment glucose and alkaline and produce H₂S. In Urea test, *Proteus mirabilis* has ability to produce urease and this enzyme will break down urea marked color change from yellow to medium pink as in figure 3 and 4. Eight samples (33%) were positively identified with *Proteus sp* then proceed to PCR analysis.

Eight *Proteus sp*-positive samples were confirmed by PCR. The result of PCR revealed only 3 out of 8 samples showed the band at 532bp. The positive control of *Proteus mirabilis* showed band at the same length. Dwiyitno (2010) explained that the PCR technique enables the identification of microorganisms specifically and rapidly in different types of food products/food samples.

Table 2. Results of biochemical tests on 24 samples of six traditional market town of Makassar

No	Sample code	Gram color	TSI A	MRVP MR	VP	SIM	C	U	G	L	S	M	note
1.	ATCC 43071	(-)	+	+	(-)	(-) Motility + H2S +	+	+	+	-	-	-	Further tests PCR
	D1	(-)	(-)	(-)	(-)	(-) Motility (-) H2S +	-	-	-	-	-	-	
	D2	(-)	(-)	(-)	(-)	(-) Motility (-) H2S +	+	+	+	-	-	-	
	D3	(-)	+	+	(-)	(-) Motility + H2S +	+	+	+	-	-	-	Further tests PCR
	D4	(-)	+	+	(-)	(-) Motility + H2S +	+	+	+	-	-	-	Further tests PCR
2.	S1	(-)	+	+	(-)	(-) Motility + H2S +	+	+	+	-	-	-	Further tests PCR
	S2	(-)	+	+	(-)	(-) Motility + H2S +	+	+	+	-	-	-	Further tests PCR
	S3	(-)	-	+	(-)	(-)	+	+	-	-	-	-	
	S4	(-)	-	+	(-)	(-)	+	-	-	-	-	-	
3.	B1	(-)	-	-	(-)	(-)	-	+	-	-	-	-	
	B2	(-)	+	+	(-)	(-)Motility + H2S +	+	+	+	-	-	-	Further tests PCR
	B3	(-)	-	-	(-)	(-)	-	+	-	-	-	-	
	B4	(-)	-	-	(-)	(-)	+	-	-	-	-	-	
4.	TL1	(-)	-	-	(-)	(-)	+	-	+	+	+	+	
	TL2	(-)	-	-	(-)	(-)	+	-	-	-	-	-	
	TL3	(-)	-	-	(-)	(-)	-	-	-	-	-	-	
	TL4	(-)	-	-	(-)	(-)	-	-	-	-	-	-	
5.	PT1	(-)	-	-	(-)	(-)	-	-	-	-	-	-	
	PT2	(-)	-	-	(-)	(-)	-	-	-	-	-	-	
	PT3	(-)	+	+	(-)	(-) Motility + H2S +	+	+	+	-	-	-	Further tests PCR
	PT4	(-)	+	+	(-)	(-) Motility + H2S +	+	+	+	-	-	-	Further tests PCR
6.	MC1	(-)	-	-	(-)	(-)	-	-	-	-	-	-	
	MC2	(-)	+	+	(-)	(-) Motility + H2S +	+	+	+	-	-	-	Further tests PCR
	MC3	(-)	+	+	(-)	(-) Motility + H2S +	-	-	+	-	-	-	
	MC4	(-)	-	-	(-)	(-)	-	-	-	-	-	-	

Note:

TSIA = *Triple Sugar Iron Agar*SIM = *Sulfur Indol Motility*MR = *Metil Red*VP = *Voges Proskauer*G = *Glucose*L = *Lactose*S = *Sucrose*M = *Mannitol*C = *cytrate*U = *Urea*

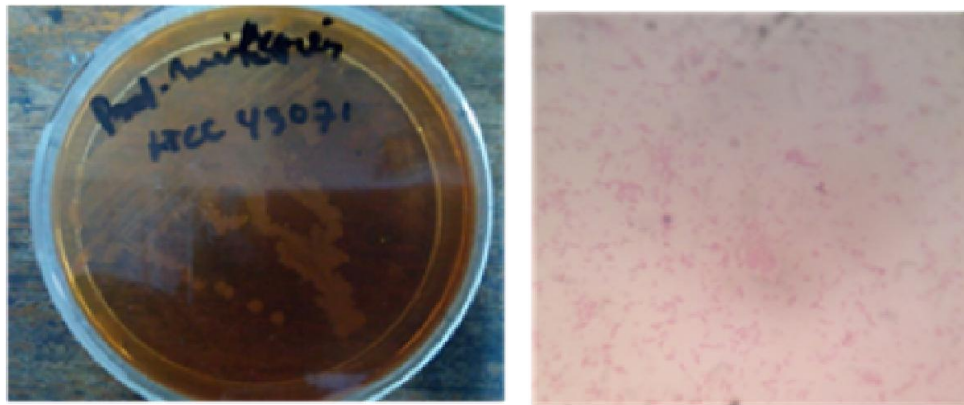


Figure 1. A. Profile *Proteus mirabilis* ATCC 43 071 on the MCA Media colorless (left) and B. Results of gram (-) staining 1000x magnification (right).

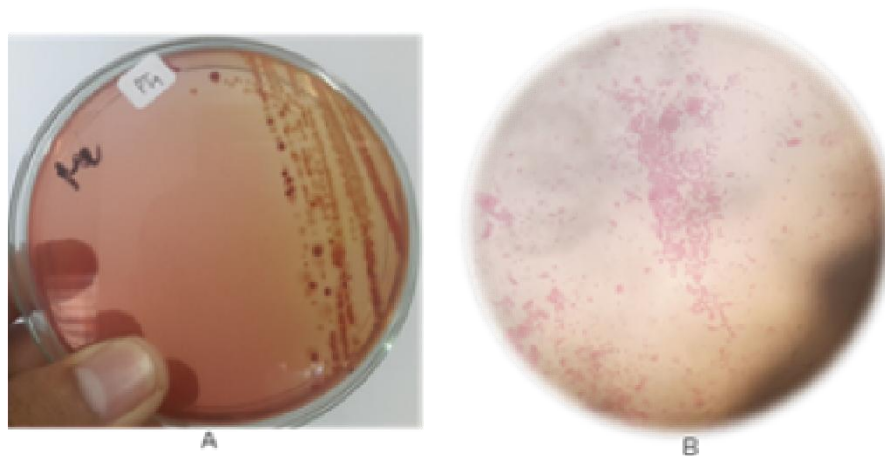


Figure 2. A. Profile colony isolates pt4 the MCA Media colorless (left) and B. Results of gram (-) staining. 1000x magnification (right).

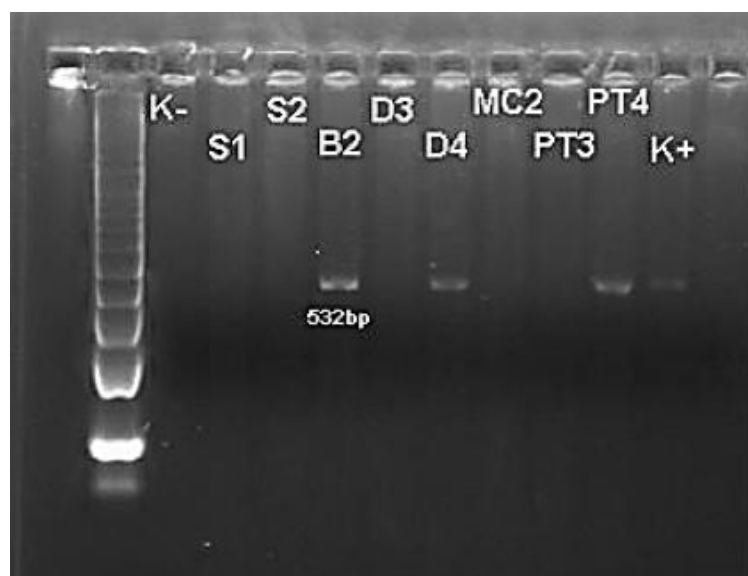


Figure 3. PCR analysis results from 8 samples of the chicken carcass (K- = H₂O, K+ = *Proteus mirabilis* ATCC 43 071)

The detected *Proteus mirabilis* contamination of the PCR method suggested that consumers have to be aware of chicken meat sold in traditional markets in Makassar. It is also necessary to improve hygiene and sanitation of the traditional market to prevent the adverse effects of *Proteus mirabilis*.

Conclusion

The results of this present study suggested that *Proteus mirabilis* was found in 3 out of 24 samples of chicken meat. Chicken meat sold in the traditional market of Makassar has already been contaminated with *Proteus mirabilis*.

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